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Short communication

Fatal toxoplasmosis and concurrent *Calodium hepaticum* infection in Korean squirrels (*Tanias sibericus*)

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Abstract

Four Korean squirrels (*Tanias siberius*) imported in Spain from People's Republic of China died 2 days after their arrival at a pet shop. They had neurological signs associated with generalized toxoplasmosis involving brain, lungs, liver, and the heart. *Toxoplasma gondii*-like tachyzoites and tissue cysts were found in organs of all four squirrels. The protozoa stained positively with *T. gondii* polyclonal antibodies and were ultrastructurally similar to *T. gondii*. *Calodium (Capillaria) hepaticum* infection was found in the liver of one squirrel.
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Keywords: *Toxoplasma gondii*; *Calodium hepaticum*; Immunohistchemistry; Tissue cysts; Tachyzoites; Electronmicroscopy

1. Introduction

Toxoplasma gondii infection is common in many species of animals including humans (Dubey and Beattie, 1988). *T. gondii* infections have been reported in the eastern gray squirrel (*Sciurus carolineusis*) (Walton and Walls, 1964; Van Pelt and Dieterich, 1972; Roher et al.,1981; Dubey et al., in press) and the California tree squirrel (*S. griseus*) (Soave and Lennette, 1959). This is the first report of fatal toxoplasmosis in the Korean squirrel (*Tanias sibericus*).

Calodium hepaticum (syn. *Capillaria hepatica*) parasitizes livers of many rodents and lagomorphs but also other mammals (Landolfi et al., 2003; Redrobe and Patterson-Kane, 2005). Rats are considered the main reservoir for this nematode, which can cause zoonotic infections (Juncker-Voss et al., 2000; Spratt and Singleton, 2001). *C. hepaticum* infection was found in one of these four squirrels with toxoplasmosis.

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2. Material and methods

Four Korean squirrels imported from Peoples Republic of China developed neurologic signs and dyspnea 2 days after arrival at a pet shop in Cordova, Spain and died. They had not been quarantined. Necropsy examination was performed on all four animals.

Tissues specimens were fixed in 10% formalin and sections were processed routinely, sectioned, stained with hematoxylin and eosin (HE), and examined microscopically. Portions of lung and heart were processed for transmission electron microscopy (TEM). De-paraffinized sections were stained with polyclonal sera to *T. gondii* and *Neospora caninum* as described previously (Lindsay and Dubey, 1989; Dubey et al., 2001). Selected sections were exposed to BAG1 antibodies against *T. gondii* bradyzoites (McAllister et al., 1996).

Specimens of lungs and livers of all four squirrels, previously fixed in 10% formalin, were diced to obtain 1 mm^3 cubes and routinely prepared for transmission electron microscopy (TEM). The samples were fixed in 1% osmium tetroxide, dehydrated in acetone and embedded in araldite. 50 nm sections were contrasted with lead citrate and uranyl acetate, and examined under a Philips CM10 electron microscope.

3. Results

3.1. Lesions

The carcasses of squirrels appeared to be in good physical condition. The lungs and livers were congested. Clear microscopic lesions were predominantly found in the liver, lungs, and the heart (Table 1). The pulmonary lesions were characterized by infiltration of alveolar septa by mononuclear cells, presence of activated macrophages in alveoli and proliferation of peribronchiolar lymphoid tissue and perivascular edema. Groups of protozoal tachyzoites were present in alveoli, mostly in macrophages.

The hepatitis was characterized by multifocal necrosis of hepatocytes, and periportal infiltration of mononuclear cells (Fig. 1). Numerous protozoa were present in hepatocytes and Kupffer cells.

Table 1
Lesion intensity, *T. gondii*, and *Calodium hepaticum* (*Capillaria hepatica*) infections in squirrels from Spain

	Squirrel number			
	1	2	3	4
Brain				
T. gondii	+	+	+++	+
Gliosis	−	−	+	−
Heart				
T. gondii	+++	+	++	+
Myocarditis	+	++	+	−
Lung				
T. gondii	+++	+	+	+
Peribronchial lymphoid tissue proliferation	+++	+++	−	−
Fibrin in alveoli	++	+	−	−
Liver				
T. gondii	+	+	−	−
Hepatocyte necrosis	+	++	++	−
Hepatitis	+++	+++	++	−
Steatosis	+	+	+	++
Capillaria hepatica	−	−	+++	−
Spleen				
Red pulp				
T. gondii	+++	+	+	−
Fibrin deposits	+	++	+	−
Necrosis	+	+	−	−
Splenomegaly	+++	+++	++	+
White pulp				
T. gondii	+	+	−	−
Necrosis	+	+	+	−

Degrees of intensity: (−) absence; (+) low, (++) moderate, (+++) intense.

3.2. Protozoa

T. gondii-like tachyzoites and tissue cysts were identified in all tissues with lesions (Table 1). Tissue cysts were seen in the lungs, heart, and the brain.

Ultrastructurally, *T. gondii*-like tachyzoites and tissue cysts (Figs. 2 and 3) were found in endothelial cells, type I pneumocytes, alveolar macrophages, monocytes and interstitial macrophages (Fig. 2). Tissue cysts had a well-defined cyst wall enclosing numerous bradyzoites (Fig. 3). The bradyzoites had numerous amylopectin granules, a terminal nucleus, and electron-dense rhoptries. The tachyzoites had no

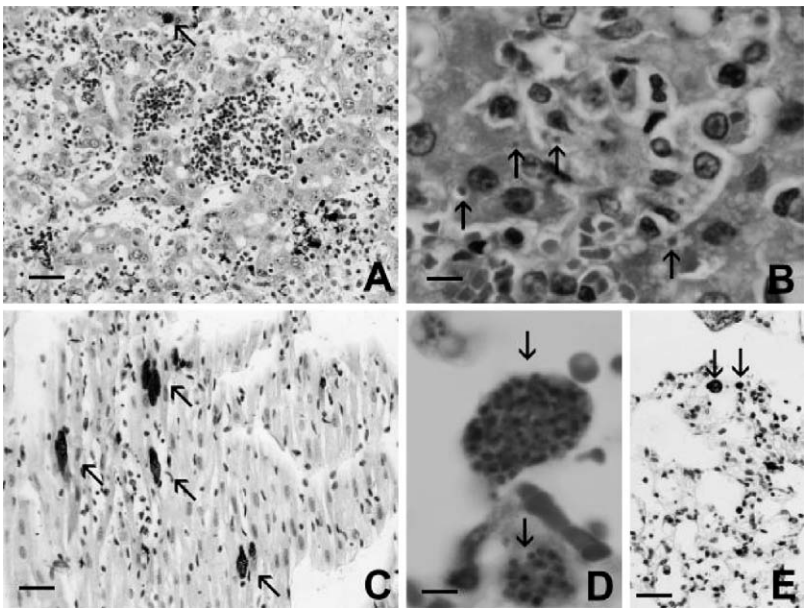

Fig. 1. Lesions and *T. gondii* in sections of tissues of squirrels. (A) Focal hepatitis with mononuclear cell infiltration and a group of tachyzoites (arrow). Immunohistochemical staining (IHC) with polyclonal *T. gondii* antibodies. Bar = 25 um. (B) Focal hepatic necrosis with individual tachyzoites (arrows). H and E stain. Bar = 5 um. (C) Several *T. gondii* tissue cysts (arrows) in the myocardium. IHC staining withh polyclonal *T. gondii* antibodies. Bar = 5 um. (D) Two *T. gondii* tissue cysts (arrows) in alveoli. H and E stain. Bar = 4 um. (E) Two *T. gondii* tissue cysts (arrows) in alveolus of lung. IHC staining with BAG 1 antibodies. Bar = 30 um.

amylopectin, a centrally located nucleus and the rhoptries were electron-lucent. Tissue cysts were seen in myocardial cells, endothelial cells and lung macrophages.

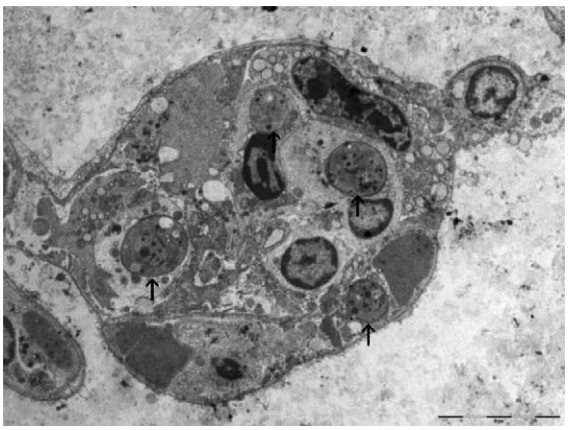

Fig. 2. TEM of intracellular tachyzoites (arrows) in monocytes in septum of lung of squirrel no. 1. Bar = 5 um.

3.3. Immunohistochemical staining

The protozoa in all tissues with lesions reacted positively with antibodies to *T. gondii* but not with

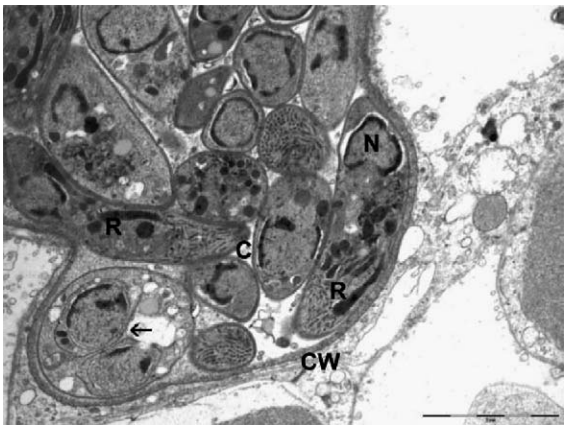

Fig. 3. TEM of a *T. gondii* tissue cyst in an endothelial cell of lung of squirrel no 1. Note a clear tissue cyst wall (CW), enclosing bradyzoites that have a conoid (C), a terminally located nucleus (N), and electron-dense rhoptries (R). Also note a dividing organism (arrow). Bar 2 μm.

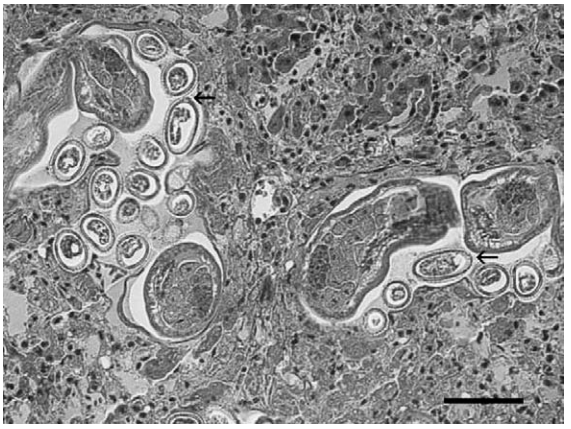

Fig. 4. Section of liver of squirrel no. 3. Note adults and bioperculate eggs (arrows) of *C. hepaticum* in the hepatic parenchyma. HE. Bar = 50 um.

antibodies to *N. caninum*. Tissue cysts reacted positively with BAG-1 antibodies that are bradyzoite-specific (Fig. 1). An unusual observation was the presence of numerous BAG1 positive tissue cysts in the heart of animal no. 2. Few tissue cysts were present in the lungs and liver of animal no. 1.

3.4. Nematode infections

In the liver of one of the animals, *C. hepaticum* adults and their characteristic bioperculate eggs (Fig. 4), were associated with hepatitis characterized by infiltration of macrophages, lymphocytes, eosinophils and plasma cells.

4. Discussion

In our case, as in the previous cases of acute toxoplasmosis in squirrels (Soave and Lennette, 1959; Roher et al., 1981; Van Pelt and Dieterich, 1972), the main signs were neurologic, clinically, indistinguishable from those seen in rabies virus infections.

Finding of numerous well-developed tissue cysts accompanied by reaction in several organs, in particular the heart, suggests that the animals had chronic infection with *T. gondii.* It can be concluded that the stress during transport would most likely the trigger for the relapse in these animals. These cases have public health relevance as *T. gondii* has been found in the saliva of acutely ill animals and transmission to humans by the bite of animals with acute toxoplasmosis remains a possibility (Dubey and Beattie, 1988).

Infection by *C. hepaticum* has been reported in several species of rodents (Landolfi et al., 2003; Redrobe and Patterson-Kane, 2005), but appears to be the first record in the Korean squirrel. *C. hepaticum* infection is zoonotic, although human infections are rare (Juncker-Voss et al., 2000).

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